Apparatus for Analyzing ~~Substantially Undiluted Samples of~~ Biologic Fluids

This application is a divisional of, and claims priority under 35 U.S.C. §120 from United States Patent application serial number 09/255,673 filed Februuary 23, 1999, and claims the benefit of United States Provisional Patent application serial number 60/077,210 filed March 7, 1998.

BACKGROUND OF THE INVENTION

1. Technical Field

The present invention relates to apparatus for analyzing biologic fluid samples in general, and to apparatus capable of performing analyses in a variety of disciplines including hematology, biologic chemistry, immunochemistry, serology, immunology, and urinalysis in particular.

2. Background Information

Historically, biologic fluid samples such as whole blood, urine, cerebrospinal fluid, body cavity fluids, etc. have had their particulate or cellular contents evaluated by smearing a small undiluted amount of the fluid on a slide and evaluating that smear under a microscope. Reasonable results can be gained from such a smear, but the accuracy and reliability of the data depends largely on the technician's experience and technique. In addition, although biologic fluid sample smears are widely used for evaluation purposes, their labor-intensive natures makes them generally not favored for commercial applications.

Another known method for evaluating a biologic fluid sample involves diluting a volume of the sample, placing it within a chamber, and manually evaluating and enumerating the constituents within the diluted sample. Dilution is necessary if there is a high concentration of constituents within the sample, and for routine blood counts several different dilutions may be required because it is impractical to have counting chambers or apparatus

which can examine variable volumes as a means to compensate for the disparities in constituent populations within the sample. In a sample of whole blood from a typical individual, for example, there are about 4.5 x 10^6 red blood cells (RBC's) per microliter (µl) of blood sample, but only about 0.25 x 10^6 of platelets and 0.007 x 10^6 white blood cells (WBC's) per µl of blood sample. To determine a WBC count, the whole blood sample must be diluted within a range of about one part blood to twenty parts diluent (1:20) up to a dilution of approximately 1:256 depending upon the exact dilution technique used, and it is also generally necessary to selectively lyse the RBC's with one or more reagents. Lysing the RBC's effectively removes them from view so that the WBC's can be seen. To determine a platelet count, the blood sample must be diluted within a range of 1:100 to about 1:50,000. Platelet counts do not, however, require a lysis of the RBC's in the sample. A disadvantage of evaluating a whole blood sample in this manner is that the dilution process is time consuming and expensive. In addition, adding diluents to the whole blood sample increases the error probability within the sample data. Adding diluents also increases the quantity of waste material that must be disposed of upon completion of the test.

A modern method for evaluating a biologic fluid sample is impedance or optical flow cytometry. Flow cytometry involves circulating a diluted fluid sample through one or more small diameter orifices, each employing an impedance type or an optical type sensor ~~which~~ that evaluates the constituents as they pass through the orifice in single file. Using the example of whole blood again, the sample must be diluted to mitigate the overwhelming number of the RBC's relative to the WBC's and platelets, and to provide adequate cell-to-cell spacing so that individual cells may be analyzed. Although more expedient and consistent than the above described methods, flow cytometers also possess numerous disadvantages. Some of those disadvantages stem from the plumbing required to carry the sample to, and the fluid controls necessary to control the fluid flow rate through, the sensor means. The precise control of this flow is essential to the cytometer's accurate operation. The plumbing within flow cytometers can and often does leak, potentially compromising the accuracy and the

safety of the equipment. The fluid flow controls and dilution equipment require periodic recalibration. In fact, the need for recalibration illustrates the potential for inaccurate results and the undesirable operating costs that exist with many presently available hematology analyzers which use flow cytometers and/or impedance orifices. The volume of reagents required to satisfy large dilution ratios increases the operating cost initially by virtue of the reagent purchase price and subsequently because of the additional waste disposal costs.

Another modern method for evaluating biologic fluid samples is one that focuses on evaluating specific subtypes of WBC's. This method utilizes a cuvette having an internal chamber about 25 microns thick with one transparent panel. A laser beam of light passing through the transparent panel scans the cuvette for WBC's. Reagents added to the sample cause each WBC to fluoresce when excited by the laser beam. The fluorescing of the particular WBC's provides an indication that particular types of WBC's are present. Because the red blood cells form a partly obscuring layer in this method, they cannot themselves be enumerated or otherwise evaluated, nor can the platelets.

There are a multitude of methods for determining the presence of soluble constituents, such as chemical components, antibodies, etc., within a sample of biologic fluid such as urine, plasma, or serum. Most of the methods require dilution of the sample and the addition of one or more reagents to the sample. Other methods require a small, but carefully metered, drop of biologic fluid sample be added to a piece of reactive film. Different analytical instruments are usually required for each method of analysis, and those instruments are expensive not only in terms of initial capital cost but also in terms of the maintenance over the life of the various instruments, and the operator training necessary to properly staff the various instruments. The operator function can vary considerably from instrument to instrument, thereby increasing the complexity of the operator training and the potential for operator error. To date, because of the widely differing requirements of the various tests, there is not a single instrument platform which will perform cross-discipline tests, most especially tests of